REMARKS

Claims 1-15 and 23-26 are pending in the present application. Reconsideration of the present application in view of the following remarks is respectfully requested. Upon entry of these amendments, claims 1-10, 13-15 and 23-27 will be pending in this application. Claims 1, 6, 9, 25 and 26 have been amended. No new matter has been added by these amendments. Support in the specification for amended claim 1 is found in the specification at page 5, line 9 to page 8, line 3; Example 1. Support for amended claim 6 is found at page 17, line 1. Support for amended claim 9 is found at page 8, lines 17-21. Support for claims 25 and 26 is found at page 4, lines 17-26; page 17, lines 10-12 and 20-21. Finally, support for new claim 27 is found at page 16, lines 23-25.

I. CLAIM OBJECTIONS

The Examiner has objected to claims 25 and 26 because these claims recite the limitation "agent" whereas the Examiner believes the claims list more than one agent. Applicants have amended claims 25 and 26 to clarify that the differentiating agent comprises the *combination* of the recited growth factors. Applicants respectfully state that this amendment moots the Examiner's objection regarding these claims.

II. CLAIM REJECTIONS UNDER 35 U.S.C. § 103

The Examiner has rejected claims 1-15 and 23-26 under 35 U.S.C. §103(a) as being obvious over Hoshimaru, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93:1518-1523 (1996) and Prasad, *et al.*, *In Vitro Cell Dev.* 30A:596-603 (1994) in view of Boss, *et al.* U.S. Pat. No. 5,411,883 (1995) and Gallyas, *et al.*, *Neurochem. Res.* 22(5):569-575 (1997), and further in view of Casper *et al.*, *J. Neurosci. Res.* 30(2):372-81 (1991) and Nikkah *et al.*, *Exp. Brain Res.* 92(3):516-523 (1993). The Examiner bases this conclusion on the belief that Hoshimaru teaches the immortalization of rat neuronal progenitor cells wherein the expression of the growth-promoting gene *v-myc* is conditionally driven by a tetracycline-controlled transactivator and a human CMV promoter, while Prasad, *et al.* discloses the isolation of an immortalized dopamine-producing nerve cell line derived from fetal rat mesencephalic tissue transfected with an oncogene. The Examiner further believes that Boss, *et al.* teaches the isolation and monolayer culture of human mesencephalon neural progenitor cells, while Gallyas, *et al.* discloses the characterization of mouse immortalized neuronal cell lines by measuring the concentration of

various neurotransmitters. The Examiner further believes that Casper *et al.* teaches that EGF enhances the survival of rat embryonic mesencephalon dopamine neurons, and that Nikkah *et al.*, teaches that PDGF promotes the survival of rat and human dopaminergic neurons in culture. The Examiner believes that it would have been obvious to modify the teachings of Hoshimaru, *et al.* and Prasad, *et al.* with the substitution of human mesencephalic cells as taught by Boss, *et al.* The Examiner further believes it would be obvious to characterize immortalized human mesencephalic cells as described by Gallyas, *et al.* because GABA and dopamine are neurotransmitters of interest. Applicants respectfully disagree with the Examiner's assertions and conclusions, and traverse this ground of rejection.

A. The Law of Obviousness

The initial burden is on the Examiner to make out a *prima facie* case of obviousness. MANUAL OF PATENT EXAMINING PROCEDURE (MPEP) § 2142, at 2100-96. To establish a *prima facie* case of obviousness, three criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge available to one of skill in the art to combine the references. Second, there must be a reasonable expectation of success. Finally, the prior art references, when combined, must teach or suggest all of the claim limitations. MPEP § 2142, at 2100-97.

Applicants respectfully assert that the combination of references cited by the Examiner fails at least two of these criteria. First, the combination(s) fails to teach every element of the claimed invention. Second, the Examiner has proffered no evidence that the cited combination provides a reasonable expectation of success in practicing the present invention. Thus, the cited references cannot in combination render the invention obvious, and consequently the Examiner has not made out the required *prima facie* case of obviousness.

B. The Combination of References Does Not Teach Every Limitation

The combination of *six* references cited by the Examiner fails first because it does not teach every limitation of any of the claims.

Applicants have amended claim 1 to more clearly recite the method steps involved. Claims 1-5, 9, 10 and 25-27 are not obvious over the cited references because none teaches the use of FGF-2, EGF and PDGF together. Moreover, none of the cited references teaches the order of the culturing steps of claim 1. Hoshimaru *et al.* plates cells in a first

medium, replaces the medium with a second medium, *then* transfects (*see* p. 1519). Prasad *et al.* transfects cells first, then plates in a first medium, and finally plates in a second medium (*see* pp. 596-597). Boss do not transfect the cells at all.

Claim 6, directed to conditionally-immortalized progenitor cells, is not obvious over the cited references. Contrary to the Examiner's assertions, Boss, *et al.* does not describe the production of monolayers of cells as recited by the current invention. Applicants are aware that Boss *et al.* uses the word "monolayer" to describe the cell cultures disclosed therein. However, Boss, *et al.* specifically states that "[g]ross examination of typical neuron progenitor cell 'monolayer' cultures reveals interconnected three-dimensional structures, rather than the usual two-dimensional monolayer observed with most cell lines."[1] Col. 6, ll. 4-7. In contrast, claim 6 of the instant application clearly recites "*adherent* monolayers" - that is, a two-dimensional layer of cells. The Examiner has not explained how cells that grow in clumps are the equivalent of cells that grow in sheets. Again, in making out a *prima facie* case of obviousness, it is up to the Examiner to *factually* support such a conclusion. *MPEP* § 2142, at 2100-96. A reference must be cited for what it *fairly* suggests. *In re Burkel*, 201 U.S.P.Q. 67 (C.C.P.A. 1979). Here, the description of the "monolayers" in Boss *et al.* suggests that they are not adherent monolayers, and are substantially different from those described in the instant application.

Claims 13-15, 23 and 24 are also not obvious over the cited art. The Examiner has not made clear which reference is being applied to assert the obviousness of claims to cells, but Applicants will assume it is the Hoshimaru *et al.*, Prasad *et al.* and Boss *et al.* references. Hoshimaru *et al.*, Prasad *et al.* disclose rat cells. Applicants wish to point out that rat cells are compositions of matter that are substantially different from human cells. The two come from a completely different source, have different biochemical markers, and react differently to culture and proliferation conditions. Rat cells are considered substantially different than human cells by those in the art. The Examiner has provided no reference suggesting or teaching what modifications of the rat neuronal cells should be performed to arrive at the claimed human neuronal cells.

The Boss *et al.* reference does disclose proliferating human neural precursors. The Examiner states that "it would have been obvious . . . to substitute the immortalized rat

---

[1] Indeed, Boss *et al.* here place "monolayer" in quotes to indicate that the cultures are not monolayers as persons of skill in the art recognize them.

neuronal progenitor cells as taught by Hoshimaru *et al.* and Prasad *et al.* with human mesencephalon neuron progenitors as taught by Boss *et al.*" Action, at p. 5. This fails to explain how the cell claims of the instant invention are obvious, however, because the fact that one cell may substitute for another does *not* mean that the second is obvious in light of the first. Applicants note, too, that the Examiner has not suggested that the cells of Boss *et al.* could be conditionally-immortalized.

It appears that the Examiner may have confounded the product disclosed in the references with the process used to make them. For example, the Examiner states that "it would have been obvious . . . to substitute the immortalized rat neuronal progenitor cells as taught by Hoshimaru et al and Prasad et al with human mesencephalon neuron progenitor cells as taught by Boss et al." (Emphasis added.) In essence, the Examiner argues that the method of Hoshimaru makes the claimed conditionally-immortalized progenitor cells, and the resulting differentiated cells, obvious. In making this argument, the Examiner follows essentially the same obviousness analysis disallowed in *In re Deuel*, 51 F.3d 1552 (Fed. Cir. 1995) (method of making a DNA molecule cannot render obvious the DNA molecule itself). Thus, the general method of making immortalized *rat* neuronal progenitor cells taught in Hoshimaru *et al.* cannot render obvious the claimed conditionally-immortalized human neuronal progenitor cell itself.

Thus, the cells disclosed in Hoshimaru *et al.*, Prasad *et al.* and Boss *et al.* references do not render the cell of the instant invention, or of claims 6, 13-15, 23 and 24, obvious.

C. The Cited Combinations of References Provide No Reasonable Expectation of Success of Practicing the Claimed Invention

The Examiner has not established that the combination of references provides a reasonable expectation of success in practicing the present invention. The Examiner asserts that "[i]t would have been obvious to one of ordinary skill in the art . . . to substitute the immortalized rat neuronal progenitor cells as taught by Hoshimaru et al and Prasad et al with human mesencephalon neuron progenitor cells as taught by Boss *et al.*" Action, at p. 5. However, the Examiner has provided no basis to support a reasonable expectation that the method of Hoshimaru, *et al.* would work using human mesencephalic neuronal progenitor cells.

For example, the Examiner states that "mesencephalon cells are easy to transfect." Action, at p. 5. However, ease of transfection does not mean that the human precursors described in Boss *et al.* would subsequently proliferate, differentiate, and mature into neurons under the

conditions outlined in Hoshimaru, *et al.* Indeed, the Hoshimaru *et al.* reference itself does not suggest that the immortalizing vector can be used in human cells; it merely suggests the immortalization of other cells from the *rat* brain (*see* p. 1522, right column).

The Examiner also cites the purported action of EGF and PDGF in the promotion of mesencephalon cell survival to support a reasonable expectation of success. Action, at p. 5. The Examiner cites Casper *et al.* as teaching that EGF enhances the survival of rat dopamine neurons, and Nikkah *et al.* as teaching that PDGF promotes survival of rat and human mesencaphalic neurons in culture. Applicants have amended claim 1 to recite that the second second serum-free growth medium comprises FGF-2, EGF and PDGF; neither Nikkah *et al.* nor Casper *et al.* teach the use of these three factors together. Applicants respectfully also point out that both references demonstrate the effect on the *survival* of *neurons*, not on the proliferation of conditionally-immortalized human mesencephalon progenitor cells. Moreover, Hoshimaru *et al.* itself argues against the Examiner's position, as the authors never used EGF and PDGF. There would thus not be a reasonable expectation of success using the cells disclosed in Boss *et al.*, in the method of Hoshimaru *et al.*, including the addition of EGF and PDGF as taught by Casper *et al.* and Nikkah *et al.* (indeed, the references do not suggest the use of FGF-2, EGF and PDGF together).

The assertion of obviousness made by the Examiner is, therefore, essentially that it would be obvious to *try* substituting the rat cells used in Hoshimaru, *et al.* with human cells. However, "obvious to try" is an improper basis for a §103(a) rejection. *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988). Thus, the invention as embodied in the present invention cannot be rendered obvious by Hoshimaru, *et al.* in combination with the other cited references.

In addition to the above, there are a plethora of differences between the methodologies of the cited references, differences that demonstrate that cell culture methods are highly specific, and that procedures from one method cannot be routinely ported to another. For example, Hoshimaru, *et al.* uses DMEM, a minimal medium, and Ham's F-12, a defined medium, with N2 supplement. *See* p. 1519, col. 1, ¶ 3. Prasad, *et al.* uses only the defined medium MCDB-153, which contains different ingredients than DMEM and F-12, followed after one year by F12 medium. *See* p. 597, col. 2, ¶ 2. Prior to the present invention, those of ordinary skill in the art would have had no basis for determining which elements of the Hoshimaru, *et al.* or Prasad, *et al.* teachings to retain, and which to alter, for use with *human* mesencephalic cells, and the cited references do not provide any guidance.

Furthermore, the culture of the cell lines as taught by Prasad, *et al. requires* the use of tissue culture dishes precoated with a specialized substrate consisting of bovine serum albumen, fibronectin and collagen. *See* p. 597, col. 2, ¶2. There is no suggestion in Prasad, *et al.* that this surface would be necessary—or even useful—for the proliferation of mesencephalon progenitor cells. Rather than teaching that the cells are standard and the methods are generally applicable, Prasad *et al.* effectively teaches cells that are unique and methods that are specialized for these cells. Conversely, the cells and methods claimed by Applicants do *not* require such a specialized culture surface. Consequently, there can be no expectation that, as the Examiner suggests, one could substitute the cells of Boss *et al.*

Boss, *et al.* and Gallyas, *et al.* do not remedy these deficiencies. Boss, *et al.* is directed to the isolation and proliferation of nonimmortalized human neuron progenitor cells, while Gallyas is directed to the characterization of immortalized mouse neural cell lines by measuring the concentration of certain neurotransmitters. Neither reference discloses the Applicants' conditions suitable for producing conditionally immortalized human mesencephalic neural progenitor cells; thus, their citation in combination with Hoshimaru, *et al.* and Prasad, *et al.* cannot render the present invention obvious.

The Examiner again cites Gallyas *et al.* as teaching the characterization of mouse immortalized neuronal cell lines by measuring the concentration of various neurotransmitters such as GABA and dopamine. Gallyas, *et al.* is irrelevant to any of the claims because none of the claims are directed to the *characterization* of immortalized cell lines. Applicants respectfully request that the Examiner explain more fully the exact claims against which the Examiner cites Gallyas *et al.* and the limitations in those claims the Examiner believes this reference provides that the other cited references do not teach. Applicants respectfully state, again, that Gallyas *et al.* teaches nothing relevant to any of the pending claims, and cannot combine with the other cited art to demonstrate that the present invention is obvious.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks. No fee is believed due. If a fee is required in connection with this Response, please charge Pennie & Edmonds LLP Deposit Account Number 16-1150 for the appropriate amount.

Respectfully submitted,

By: Lawrence S. Graham Reg. No. 49,020

Date June 19, 2002 Anthony M. Insogna 35,203

Anthony M. Insogna (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

EXHIBIT A
MARKED VERSION OF THE CLAIMS
U.S. PATENT APPLICATION SERIAL NO. 09/134,771

1. (Amended three times) A method for producing a conditionally-immortalized human mesencephalon neural [precursor] progenitor cell, comprising:

(a) [transfecting] plating human mesencephalon cells [plated] on a first surface and in first growth medium that permits proliferation;

(b) transfecting said progenitor cells with DNA encoding a selectable marker and an externally regulatable growth-promoting protein; and

[(b)](c) selecting an adherent monolayer of the transfected cells on a second surface and in a second serum-free growth medium that permits attachment and proliferation, wherein the second serum-free growth medium comprises [EGF or PDGF] FGF-2, EGF and PDGF, and therefrom producing a conditionally-immortalized human mesencephalon cells in which the growth-promoting protein is regulated by an external factor, such that suppression of the growth promoting protein results in differentiation of the cell into a [neurons] neuron.

6. (Twice amended) A conditionally-immortalized human mesencephalon neural [precursor] progenitor cell capable of differentiation into neurons, wherein the cell is transfected with DNA encoding a growth-promoting protein that is regulated by an external factor, such that suppression of the growth-promoting protein results in differentiation of the cell into a neuron, and wherein the cell is polygonal and grows as an adherent monolayer.

9. (Amended) A method for producing a [neurons] neuron, comprising culturing a cell produced according to claim 1 in the presence of at least one differentiating agent under conditions that inhibit expression of the growth-promoting gene.

25. (Amended) The method of claim [12] 9, wherein the differentiating agent [is] comprises the combination of forskolin, GDNF and CNTF.

26. (Amended) The method of claim [12] 9, wherein the differentiating agent [is] comprises the combination of forskolin, GDNF, CNTF, IGF-1 and BDNF.

EXHIBIT B
CLEAN VERSION OF THE CLAIMS THAT WILL BE PENDING UPON ENTRY OF THE PRESENT AMENDMENT
U.S PATENT APPLICATION SERIAL NO. 09/134,771

1. (Amended three times) A method for producing a conditionally-immortalized human mesencephalon neural progenitor cell, comprising:

(a) plating human mesencephalon cells on a first surface and in first growth medium that permits proliferation;

(b) transfecting said progenitor cells with DNA encoding a selectable marker and an externally regulatable growth-promoting protein; and

(c) selecting an adherent monolayer of the transfected cells on a second surface and in a second serum-free growth medium that permits attachment and proliferation, wherein the second serum-free growth medium comprises FGF-2, EGF and PDGF, and therefrom producing a conditionally-immortalized human mesencephalon cells in which the growth-promoting protein is regulated by an external factor, such that suppression of the growth promoting protein results in differentiation of the cell into a neuron.

2. The method of claim 1 wherein the first and second surfaces are independently selected from the group consisting of substrates comprising one or more of a polyamino acid, fibronectin, laminin or tissue culture plastic.

3. The method of claim 1 wherein the growth-promoting gene is an oncogene.

4. The method of claim 3 wherein the oncogene is v-myc.

5. The method of claim 1 wherein expression of the growth-promoting gene is inhibited by tetracycline.

6. (Twice amended) A conditionally-immortalized human mesencephalon neural progenitor cell capable of differentiation into neurons, wherein the cell is transfected with DNA encoding a growth-promoting protein that is regulated by an external factor, such that suppression of the growth-promoting protein results in differentiation of the cell into a neuron, and wherein the cell is polygonal and grows as an adherent monolayer.

7. A conditionally-immortalized human mesencephalon neural precursor cell according to claim 6, wherein the cell is capable of differentiation into dopaminergic neurons.

8. A conditionally-immortalized human mesencephalon neural precursor cell according to claim 6, wherein the cell is capable of differentiation into GABA-ergic neurons.

9. (Amended) A method for producing a neuron, comprising culturing a cell produced according to claim 1 in the presence of at least one differentiating agent under conditions that inhibit expression of the growth-promoting gene.

10. A method according to claim 9, wherein the cell is cultured in medium comprising tetracycline.

13. A neuron produced according to the method of claim 9.

14. A dopaminergic neuron produced according to the method of claim 9.

15. A GABA-ergic neuron produced according to the method of claim 9.

23. A conditionally-immortalized human mesencephalon neural precursor cell produced according to the method of claim 1.

24. A cell according to claim 23, wherein the cell is present within a clonal cell line.

25. (Amended) The method of claim 9, wherein the differentiating agent comprises the combination of forskolin, GDNF and CNTF.

26. (Amended) The method of claim 9, wherein the differentiating agent comprises the combination of forskolin, GDNF, CNTF, IGF-1 and BDNF.

27. (New) The method of claim 9 wherein said differentiating agent comprises GDNF.